Current Trends in Biotechnology and Pharmacy
Vol. 10 (4) 334-342 October 2016, ISSN 0973-8916 (Print), 2230-7303 (Online)

Stability Indicating Assay Method Development and Validation to Simultaneously Estimate Metformin Hydrochloride and Canagliflozin by RP-HPLC

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Abstract

The focus of this study is to develop and validate a HPLC method to simultaneously estimatemetformin hydrochloride and canagliflozin in bulk using GraceSmart RP-18 column (250× 4.6mm, 5µ)at 30°C.Combination of acetonitrile(ACN) and ammonium acetate bufferin the ratio of 45:55v/v with pH 4.5 was used as mobile phase with 1ml/min flow rate. It was detected by photo diode array detector at 252 nm. The retention timeobserved for metformin hydrochloride and canagliflozin were found to be 4.00 and 5.76 min respectively. The method was developed and found to be linear with correlation coefficients r^2 of 0.9993 and 0.9992 for metformin hydrochloride and canagliflozin respectively within a concentration range of 1-80µg/ml. Stability studies were performed by exposing the drugs to acidic, basic, oxidative, thermal and photolytic stress conditions with sampleswithdrawn at different time intervals. Analysis of the above samples were done by the developed method. The method to estimate metformin hydrochloride and canagliflozin in bulk drug is easy, accurate, precise and less time consuming.

Keywords: Metformin hydrochloride, Canagliflozin, RP-HPLC, Validation, Stability indicating.

Introduction

Metformin hydrochloride (Fig.1), an anti-diabetic drug is the first line oral pharmacotherapy for the cureof type 2 diabetes (1). It is chemically known as N,N-dimethyl-imido-dicarbon-imidicdiamide (2). It reduces the production of hepatic glucose. Hence, improves hyperglycemia (3). It also decreases the glucose production in the liver by activating the energy regulating enzyme Adenosine 5'-monophosphate-activated protein kinase (AMPK) which is considered a major mode of metformin action (1).

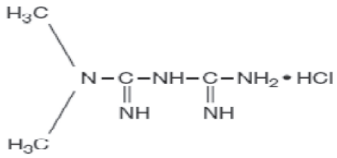

Fig.1. Structure of metformin hydrochloride

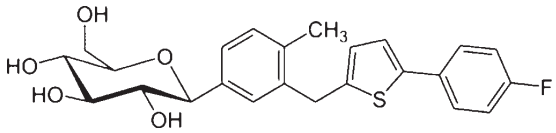

Fig. 2. Structure of canagliflozin

Canagliflozin (Fig.2) is a oral anti-diabetic agent which belongs to a newly developed class,ithas an inhibitory action on sodium-glucose co-transporter 2 (SGLT2).Itreceived approval by US FDA in March-2013 for treating the patients

having type-II diabetes. Canagliflozin is chemically named as (2S,3R,4R,5S,6R)-2-[3-[[5-(4-fluorophenyl)thiophen-2-yl]methyl]-4methylphenyl]-6(hydroxymethyl)oxane-3,4,5-triol(4, 5). SGLT2 is located in the proximal renal tubule and is majorly important for reabsorption of filtered glucose from its lumen, as SGLT2 is inhibited by canagliflozin the filtered glucose reabsorption decreases thereby lowering the kidney threshold for glucose and consequently increases the glucose excretion through urine (6).

Literature review for the simultaneous determination of metformin hydrochloride and canagliflozin by HPLC has revealed thatnot many analytical methods have been reported for the same (1, 6).By this study an easy, accurate, economic and efficient stability indicating Reverse phase-HPLC method has been developed for the simultaneous estimation of metformin hydrochloride and canagliflozin along with forced degradation studiesin bulk drug.

Materials and Methods

Chemicals and Reagents: Pharmaceutical grade reference standards for metformin hydrochloride and canagliflozin were obtained as gift samples byGlenmark Pharmaceuticals Ltd. (Sikkim, India) and MSN Life Sciences Pvt. Ltd. (Telangana, India) respectively. Methanol and acetonitrile used were of HPLC grade supplied by Finar chemicals limited, Ahmadabad. Ammonium acetate used was of analytical grade which was supplied by Spectrochem Pvt. Ltd., Mumbai. Ultra-clear water that has been used was from Millipore purification system.

***Instrumentation*:** Samples were analysed on Shimadzu HPLC (Kyoto, Japan) system controlled by LC solution software and equipped with a quaternary pump (LC-10 ADVP), Auto injector (SIL-10 ADVP), a photo diode array detector (SPD M-10A VP). The separation was done on a GraceSmart Reverse Phase18 (250 x 4.6 mm,5 µ) column.

Chromatographic Conditions: The mobile phase consisted of acetonitrile:10mM ammonium acetate buffer of pH 4.5 in the proportion of 45:55 (v/v). 0.77 gm of ammonium acetate was dissolved in 1 L of Milli-Q water and using 10 % glacial acetic acid the pH was adjusted to 4.5 ± 0.02. The resulting solution was undergone vacuum filtrationthrough a 0.45µm filter and later degassed in an ultrasonic bath for 15 minutes before use.The mobile phase was delivered with 1ml/min flow rate and the column temperature was 30ºC. The detection was carried out at 252 nm using PDA detector.The volume injected into the system was 20 microlitres having a total run time of 8 minutes.

Preparation of Standard Solution: 10mg each of metformin hydrochloride and canagliflozin working standard were accurately weighed and transferred separately into two 10 ml volumetric flasks and diluted up to the mark with methanol. From each volumetric flask, 1 ml of the stock solution was withdrawn and taken into a 10 ml volumetric flask and diluted to volume with mobile phase (MP). 1ml of the above solution was withdrawn and further diluted with mobile phase to the mark of 10ml flask to get working standard solution of 10µg/ml.

Results and Discussion

As a result, a typical chromatogram for the proposed method has been obtainedwhich is shown in Fig.3. The retention timesobserved for metformin hydrochloride and canagliflozin were found to be 4.00 min and 5.76 min respectively, having a resolution of 6.5.

Method Validation (7)*:*

System Suitability:It is carried out to evaluate the system suitability parameters (tailing factor, resolution, theoretical plates and relative standard deviation) for replicate injections The results obtained werewithin the limits and are shown in Table-1.

Specificity: It was performed to ensure that the response is due to single component only i.e. no co-elution exist between drug and excipients/ impurities. To perform this, a mixture of standard solution was prepared and peak purity was performed by PDA detector for metformin

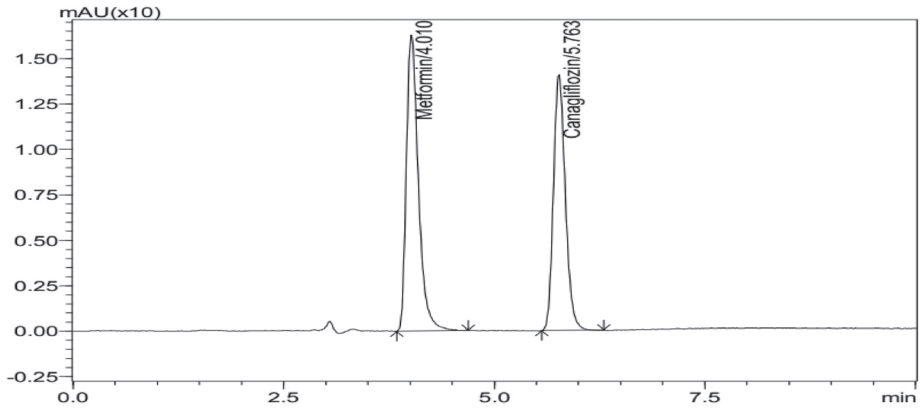

Fig.3. Optimised chromatogram of metformin hydrochloride and canagliflozin

hydrochloride and canagliflozin.The peak purity obtained was shown in Fig.4 and Fig.5.

Linearity and Range: A series of linearity solutions for the mixture of metformin hydrochloride and canagliflozin were prepared in the concentration range of 1-80 µg/ml. 20 µl of each standard was injected in triplicate and the results(chromatograms) were recorded for all the linearity standards under the optimized chromatographic conditions. The regression co-efficient for both the drugs were not less than 0.999, thus falls within the acceptance limits. The linearity graphs are shown in Figs. 6 and 7; and the results are tabulated in Table-2.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ of the developed method were determined from the standard deviation (SD) of the response and slope (m). The calculated values are tabulated in Table-3.

Accuracy: Accuracy studies have been conductedbased on the recovery of known amounts of analyteinorder to determine the proposed method accuracy by standard spiking method. The recovery of the analyte was calculated by spiking a noted amount of the

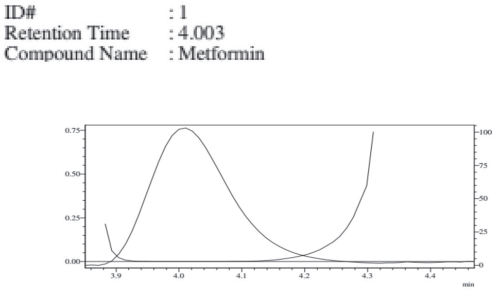

Fig. 4. Peak purity curve of metformin hydrochloride

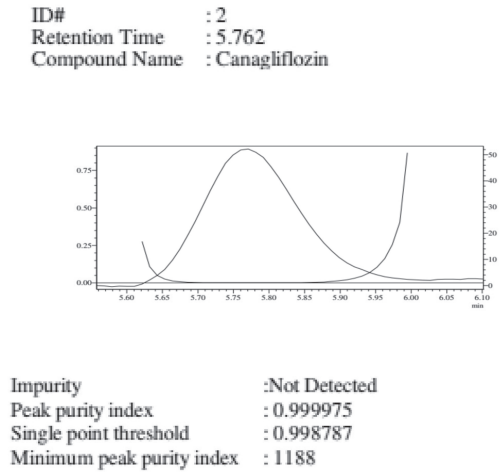

Fig. 5. Peak purity curve of canagliflozin

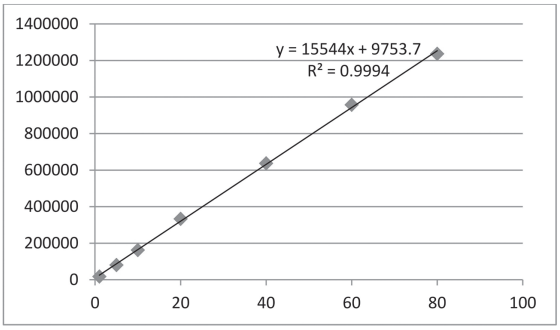

Fig. 6. Calibration curve of metformin hydrochloride

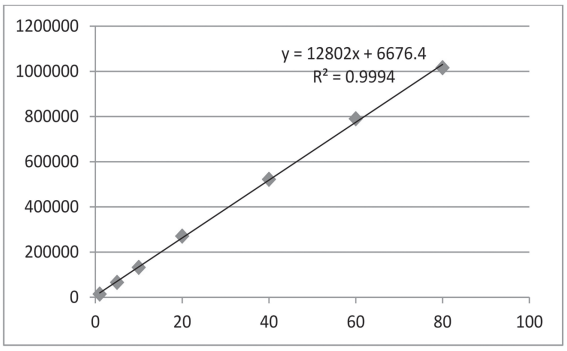

Fig. 7. Calibration curve of canagliflozin

standard drug to the pre analysed standard samples. Accuracy was performed at three known concentration levels of 80 %, 100 % and 120 % of standard concentration and sample solutionswere prepared in triplicate for each level. The % recovery was found to be within the acceptance criteria of 98-102%. The accuracy results are tabulated in Table4A and 4B.

Precision: Precision of the proposed method was carried out at three levels which were system precision, intermediate precision i.e. done in different days and different instrument and repeatability (method precision). The % RSD (result) was found to be less than 2.0% thus within the acceptance criteria. The precision results are tabulated in Table-5.

Robustness: Small variations were made in the analytical parameters of the method and robustness was studied by examining the peak area. The peak area obtained with each solution was measured and %RSD was calculated which was found to be less than 2.0%, thus within the acceptance criteria. Robustness results are tabulated in Table-6.

Forced Degradation Studies: These studies were conducted to indicate the stability indicating property of the developed method. The stressed samples of metformin hydrochloride and canagliflozin were then subjected toa peak purity test by using PDA detector. The conditions maintained for performing stress studies on metformin hydrochloride and canagliflozin are tabulated briefly in Table 7.

Acid Hydrolysis: From the stock solution (1000μg/ml) of metformin hydrochloride and canagliflozin, 1 ml was withdrawn and taken into a 10 ml volumetric flask and the volume was made up to the mark with 0.1M hydrochloric acid. The solution was then refluxed at 80 °C in a round bottom flask for 8 hours. The resultant solution was neutralized to pH 7.0 with counter base 0.1M sodium hydroxide and then diluted with mobile phase to 10μg/ml and then injected into the system. The chromatogram of acid degraded mixture after 4 hours on refluxing at 80°C is shown in Fig.8.

Alkali Hydrolysis: From the stock solution (1000 μg/ml) of metformin hydrochloride and canagliflozin, 1 ml was withdrawn and taken to a 10 ml volumetric flask and the volume was made up by using0.1 M sodium hydroxide. The solution was then refluxed at 80 °C in a round bottom flask for 8 hours. The resultant solution was neutralized to pH 7.0 with counter acid 0.1 M hydrochloric acid and then diluted with mobile phase to 10μg/ml and injected into the HPLC system.

The chromatogram of base degraded mixture after 2 hours on refluxing at 80°C is shown in Fig.9.

Oxidative Degradation: From the stock solution (1000μg/ml) of metformin hydrochloride and

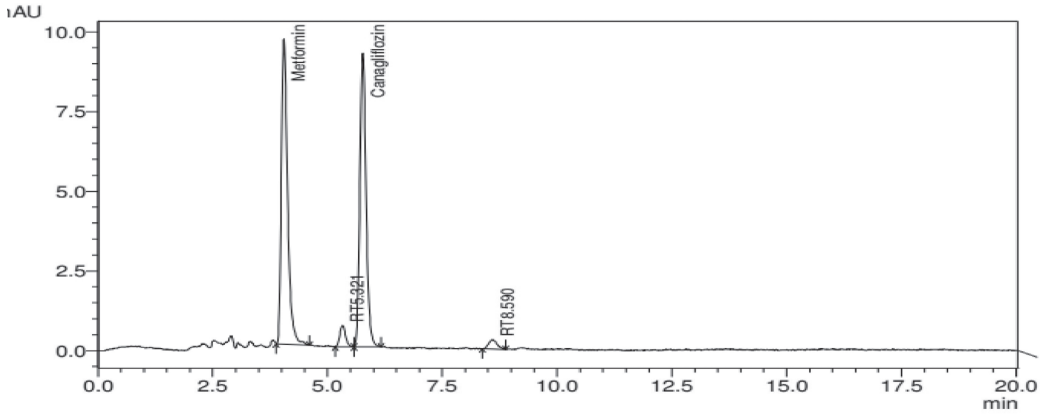

Fig.8. Chromatogram of acid degraded mixture after 4 hours (reflux at 80°C)

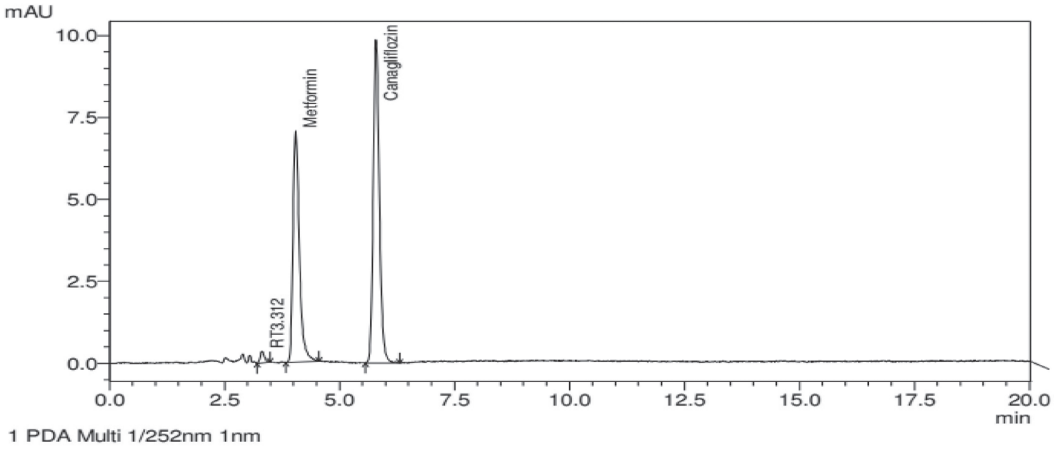

Fig.9. Chromatogram of base degraded mixture after 2 hours (reflux at 80°C)

canagliflozin, 1 ml was withdrawn and taken into a 10 ml volumetric flask and the volume was made up with 3 % v/v hydrogen peroxide solution. The resultant solution waskept aside at room temperature and diluted with mobile phaseto 10 µg/ml and injected into the system. The chromatogram of degraded mixture after 2 hrs at room temperature is shown in Fig.10.

***Thermal* Degradation:** Approximately 1 gm each of metformin hydrochloride and canagliflozin were placed separately in a petri dish and kept inside the hot air oven at 80°C for 24 hours. Samples were then collected, diluted with mobile phase to 10 µg/ml and injected into the system.

The chromatogram of mixture on exposure to heat at 24 hrs is shown in Fig,11.

Photolytic Degradation: A standard solution of 10µg/ml of metformin hydrochloride and canagliflozin in a 10 ml volumetric flask was kept outside exposed to direct sunlight for around 12 hours. The resultant solution was then injected into the system. The chromatogram of mixture on exposure to light after 12 hrs is shown in Fig.12.

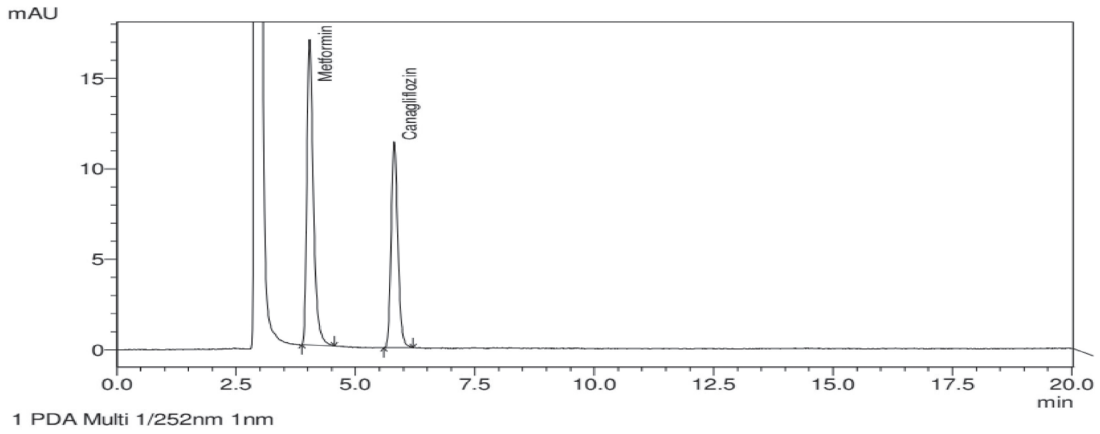

Fig.10. Chromatogram of degraded mixture after 2 hrs at room temperature

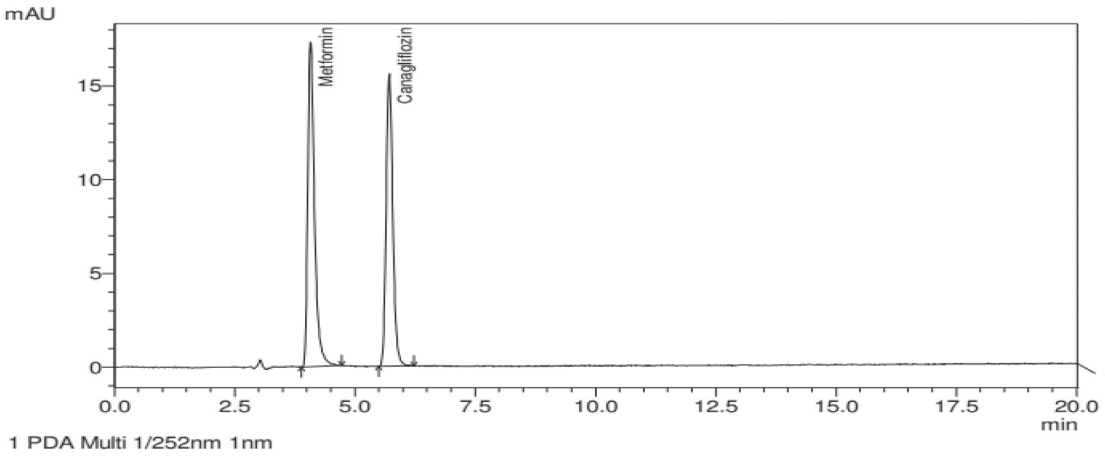

Fig.11. Chromatogram of mixture exposed to heat at 24 hrs

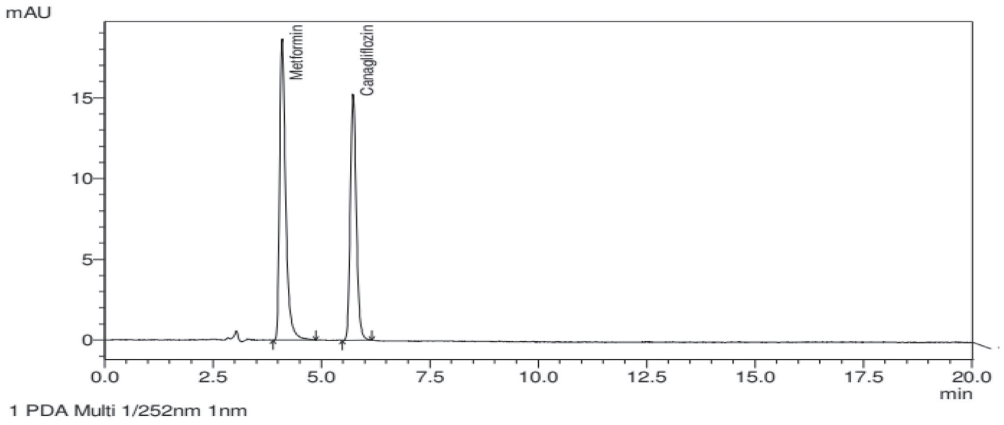

Fig.12. Chromatogram of mixture exposed to light after 12 hrs

Table 1. Summary of system suitability studies (n=6)

Parameter	Observation		Acceptance criteria
	Metformin HCl	Canagliflozin	
% RSD of retention time of 6 injections	0.10	0.10	Not more than 1.0
% RSD of area of 6 injections	0.32	0.30	Not more than 1.0
Plate count	3592.83	7264.17	More than 2000
Tailing factor	1.52	1.25	Not more than 2.0

Table 2. Data of linearity for metformin hydrochloride and canagliflozin

	Metformin hydrochloride	Canagliflozin
Regression equation	y = 15544x + 9753	y = 12802x + 6676
Correlation coefficient	0.999	0.999
Slope	15544	12802
Y-intercept	9753	6676

Table 3. Limit of detection and limit of quantitation

	Metformin HCl	Canagliflozin
LOD	0.134 μg/ml	0.124 μg/ml
LOQ	0.406 μg/ml	0.376 μg/ml

Table 4A. Accuracy results metformin hydrochloride

Spiked level	concentration (μg/ml)	Average Amount recovered(μg)	Average % recovery	%RSD
80%	18	17.95	99.39	0.10
100%	20	20.09	100.87	0.22
120%	22	21.83	98.65	0.31

Table 4B. Accuracy results canagliflozin

Spiked level	concentration (μg/ml)	Average Amount recovered(μg)	Average % recovery	%RSD
80%	18	17.93	99.19	0.27
100%	20	20.06	100.57	0.32
120%	22	22.01	100.08	0.11

Table 5. Precision studies of metformin hydrochloride and canagliflozin

Parameter	Drug	% RSD		
System Precision	Metformin hydrochloride	0.28%		
	Canagliflozin	0.27%		
Method Precision	Metformin hydrochloride	0.33%		
	Canagliflozin	0.39%		
Intermediate Precision: Different Days and Different Instruments	Day 1	Day 2	Inst 1	Inst 2
Metformin hydrochloride	0.33%	0.22%	0.31%	0.75%
Canagliflozin	0.39%	0.28%	0.27%	0.49%

*Average of 6 injections

Table 6: Robustness results of metformin hydrochloride and canagliflozi**n**

Parameter	Changed condition	% RSD	
		Metformin hydrochloride	Canagliflozin
pH [±0.2 units]	pH 4.3	0.36	0.33
	pH 4.5	0.49	0.34
	pH 4.7	0.30	0.33
Wavelength[±2 nm]	250 nm	0.44	0.10
	252 nm	0.45	0.39
	254 nm	0.31	0.25
Flow rate [±10%]	0.9 ml/min	0.27	0.25
	1.0 ml/min	0.30	0.31
	1.1 ml/min	0.25	0.18
Mobile phase [±2units]	43:57	0.45	0.42
	45:55	0.52	0.32
	47:53	0.37	0.30
Column oven temperature [±5°C]	25°C	0.24	0.16
	30°C	0.42	0.22
	35°C	0.23	0.21

*Average of 6 injections

Conclusion

The developed stability indicating assay method is found to be easy, accurate, sensitive, specific and rapid for the simultaneous estimation of metformin hydrochloride and canagliflozin and was validated in terms of accuracy,linearity, precision, limits of detection (LOD) and quantification (LOQ)according to ICH Q2(R1) guidelines. Stress studies were performed for the drug substance under acidic, alkaline, photolytic,oxidative and thermal conditions using the above optimised method. In all the conditions, the drug peaks were separated from the degradation product peak. This method can thus be applied for routine normalanalysis and stability testing of metformin hydrochloride and canagliflozin in pharmaceutical formulation like tablets and in bulk.

Table 7. Forced degradation study results of metformin hydrochloride and canagliflozin

Type of degradation	Stress Conditions	Degradation time	% of degradation observed		Peak purity
			Metformin hydrochloride	Canagli flozin	
Acid hydrolysis	0.1 M Hydrochloric acid at 80°C reflux	4 hrs	12 %	7 %	Passed
Alkali hydrolysis	0.1 M sodium hydroxide at 80 °C reflux	2 hrs	32 %	5 %	Passed
Oxidative degradation	3 % v/v Hydrogen peroxide at room temperature	2 hrs	13 %	15 %	Passed
Thermal degradation	Inside hot air oven at 80 °C	24 hrs	7 %	1 %	Passed
Photolytic degradation	Exposed to direct sunlight	12 hrs	2.7 %	2.6 %	Passed

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